Measurement of IL-22 induced expression of Reg3g and Muc1 following burn injury and ethanol exposure in mice

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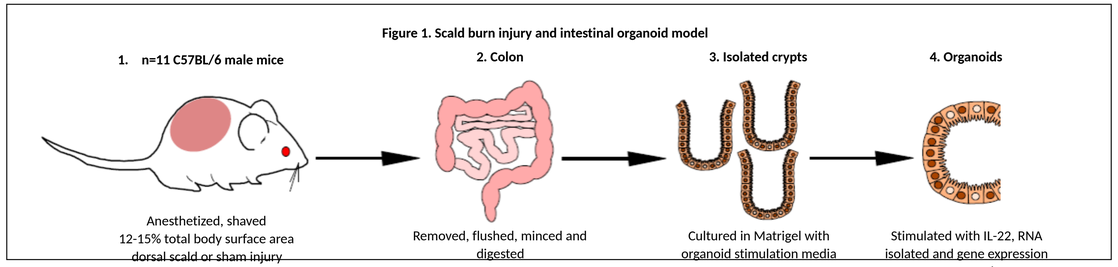
Introduction

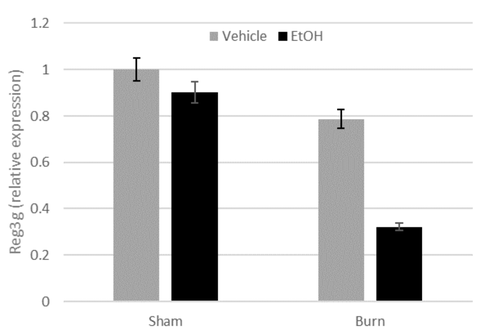
- In the United States nearly 100,000 burn injuries per year require medical attention. 20,000 of those injuries require hospitalization, and ~4,000 of those injuries result in death[1]
- Of the patients admitted for burn injuries, nearly 50% have a positive blood alcohol content and these patients have worse clinical outcomes than those who sustain injuries with no alcohol (EtOH) in their system[1]
- Following burn injury, inflammatory cytokines enter systemic circulation which increase intestinal barrier permeability and the potential for bacterial translocation and massive systemic inflammation and infection[2]
- It is well understood that Interleukin-22 (IL-22) plays a vital role in maintaining gut epithelial integrity and immune function in part by upregulating antimicrobial peptides (AMPs) including Mucin 1 (Muc1), and Regenerating islet-derived protein 3 gamma (Reg3g)[3]
- What is less understood is how the levels of these AMPs are affected following burn injury in conjunction with EtOH exposure
- The goal of this study was to examine whether burn injury in conjunction with EtOH exposure in mice affected IL-22 induction of Reg3g and Muc1 and to what degree those levels are affected

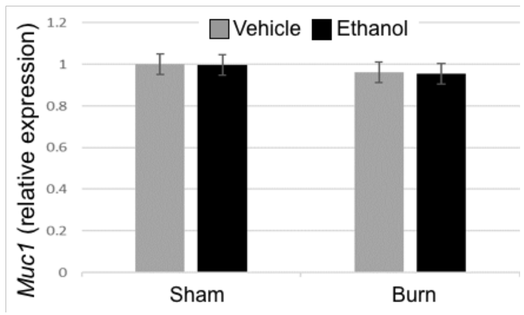
Methods



- Mice were exposed to either sham or scald burn injuries then euthanized. Colons were removed, flushed, minced and washed with washing media. Tissue was then digested with collagenase and gentamicin to liberate crypts (colon epithelial cells), which were passed through a cell strainer to isolate epithelial cells
- Isolated epithelial cells were suspended in Matrigel and incubated with conditioned media for 72 hours
- Epithelial units were then exposed to either 90% EtOH for 30 minutes or to a control vehicle. EtOH and vehicle were siphoned off, cells were stimulated with IL-22, and organoids were incubated for 24 hours
- RNA was extracted in TRIzol, reverse transcribed, and quantitative PCR was performed to assess expression of Reg3g and Muc2

Results





- PCR analysis of Reg3g revealed a statistically significant decrease in expression following EtOH exposure alone (relative expression = .902) and burn alone (relative expression = .787). EtOH administration in addition to burn injury also showed a significant decrease when compared to control (relative expression .321)
- PCR analysis of Muc1 showed a slight decrease following burn alone (relative expression = .962) and following EtoH exposure and burn injury (relative expression .960)

Conclusions

- Epithelial cells from burned mice exposed to EtOH and stimulated with IL-22 displayed significantly decreased expression of Muc1 and Reg3g compared to both burn alone and EtOH alone
- These findings may suggest the possibility of an additive nature of insult following EtOH exposure in conjunction with burn injury and a unique vulnerability to epithelial damage, bacterial translocation, and systemic inflammation
- Further research is needed to determine what role the decrease of these gut AMPs may play in the worse clinical outcomes for these patients

Acknowledgments

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References

1. Choy, K., et al. "Aberrant Inflammatory Responses in Intoxicated Burn-Injured Patients Parallel Impaired Cognitive Function." Alcohol, Elsevier, 21 Jan. 2023, www.sciencedirect.com/science/article/abs/pii/S0741832923000137?via%3Dihub.
2. Mizoguchi A. Healing of intestinal inflammation by IL-22. Inflamm Bowel Dis. 2012 Sep;18(9):1777-84. doi: 10.1002/ibd.22929. Epub 2012 Feb 22. PMID: 22359410; PMCID: PMC3366176.
3. Kolls JK, McCray PB Jr, Chan YR. Cytokine-mediated regulation of antimicrobial proteins. Nat Rev Immunol. 2008 Nov;8(11):829-35. doi: 10.1038/nri2433. PMID: 18949018; PMCID: PMC2901862.